

## Mapping QTLs for Heat Tolerance in Wheat

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### ABSTRACT

Heat stress is a major environmental stress limiting wheat productivity in most cereal growing areas of the world. In order to map and characterize quantitative trait loci controlling heat tolerance, 144 recombinant inbred lines deriving from the cross of Kauz and MTRWA116 were assessed in a greenhouse and growth chamber at 35°C. One hundred and sixty six SSR and 3 AFLP markers were used to construct a linkage map containing 18 linkage groups and covering 16 chromosomes of wheat. Using the composite interval mapping method, three QTLs were detected for heat tolerance and measured by the Fischer susceptibility index, on chromosomes 1B, 5B and 7B. The alleles of both parents contributed to heat tolerance. A large amount of explained phenotypic variances and small confidence intervals indicate that the linkage information between markers and QTLs could easily be used in breeding for heat tolerance.

**Keywords:** Heat tolerance, Linkage map, QTL, Recombinant inbred lines, Wheat.

### INTRODUCTION

Global warming, which arises from increased CO<sub>2</sub> in the atmosphere, could affect agriculture in future (Iba, 2002). Heat stress is currently a major limitation to wheat (*Triticum aestivum* L.) productivity in arid, semi-arid, tropical, and subtropical regions of the world (Fischer, 1986). Over 50 countries importing more than 20 million tons of wheat per year experience this type of stress throughout the wheat cycle (Reynolds *et al.*, 2001). Furthermore, as the world population grows exponentially, there is a need to expand productive areas into warmer climates. Consequently, the development of heat-tolerant cultivars is of major concern in wheat breeding programs (Wardlaw *et al.*, 2002). A detailed understanding of the genetics and physiology of heat tolerance and proper selection methods will facilitate the development of heat tolerant cultivars. Genetic variation for heat tolerance in wheat cultivars is well established (Al-Khatib and

Paulsen, 1990; Reynolds, 2001; Wardlaw, 1989). Exposure to higher than optimal temperatures reduces yield and decreases the quality of wheat (Fokar *et al.*, 1998; Maestri *et al.*, 2002; Wardlaw *et al.*, 2002).

Traits such as earliness, leaf rolling, plant height, early ground cover, stay green, and grain filling duration are shown to be associated with resistance to heat stress (Blum and Nguyen, 1997, Fokar *et al.*, 1998; Reynolds *et al.*, 2001). Cell membrane thermal stability, canopy temperature depression, stomatal conductance, and photosynthetic rate are all physiologically important under heat stress (Al-Khatib and Paulsen, 1984; Fokar *et al.*, 1998; Reynolds *et al.*, 1994, 2001). Yield under stress, however, is preferred by breeders for screening tolerant genotypes (Ozkan, 1998). This approach has the advantage of combining the effects of many different factors without needing to know the relative importance or the physiological basis of each factor. It should be noted that, although heat stress is almost certainly a component of drought stress, wheat germplasm that per-

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forms well under heat stress is not necessarily drought tolerant (Reynolds *et al.*, 2001).

The use of yield as a selection criterion for the development of stress tolerant varieties is prohibitive in early generations. The cost, time, labor and equipment necessary for such evaluation would easily use up a breeding program's budget. Additionally, heat stress does not happen predictably and evenly in the field and it is so difficult to separate it from drought stress as well. Hence, plant breeders look for alternative methods such as marker assisted selection (MAS). QTL mapping offers important information on the number and location of the loci which control quantitative traits and may provide a useful method for MAS (Kato *et al.*, 2000; Liu, 1998). It has been proved that heat tolerance is quantitatively inherited and continuously distributed (Blum, 1988; Yang *et al.*, 2002). To our knowledge, the only study concerning QTLs for heat tolerance is that of Yang *et al.* (2002). In it, detected two QTLs for heat tolerance measured by grain filling duration following the method of single factor analysis in an F2 population. They were, therefore, unable to map and localize the QTLs.

Nonetheless, QTL studies should be conducted in different populations and under different conditions before the results can be used in breeding programs. The objective of this study was to map and characterize quantitative trait loci controlling heat tolerance and to find the molecular markers associated with them.

## MATERIALS AND METHODS

### Plant Material

The plant population studied consisted of 144 F9 recombinant inbred lines (RILs) originating from a cross between Kauz (Jupatco F73/Blue Jay/Urest T81) and MTRWA116 (PI372129/2\*Pondera). Kauz has been developed in CIMMYT, Mexico, and is known to be tolerant to high temperature while MTRWA116 is an unreleased

experimental line from Montana State University (USA) and is considered to be thermosensitive (Fokar *et al.*, 1998; Ibrahim and Quick, 2001). The initial cross was made in December 1997, and generations were advanced by single seed descent up to F6 followed by three generations of bulk for seed increase (Butler *et al.*, 2005).

### Evaluation of Heat Tolerance

Evaluation of heat tolerance was conducted according to Yang *et al.* (2002) with some modifications. Seedlings of the 144 RILs and the parents were germinated and grown in a Metro-Mix2000® growing medium in the greenhouse at 20-25°C in special pots called Conetainer®. The experimental design was completely randomized with four replications. Plants were watered at the proper time and fertilized with a complete solution of Peter-Professional®. One week after the first anther extrusion was observed, the pots of two replications, each containing one seedling, were moved to a controlled environment chamber for heat shock. The chamber was set at 35/30 °C and 14/10 h day/night, 50/70% relative humidity and illumination of 335 μmol m<sup>-2</sup> S<sup>-2</sup>. Plants were exposed to this high temperature for three days and then moved back to the greenhouse. Since the lines' anthesis date was different, they were moved to the chamber at different times. When the color of the peduncle turned to yellow, signalling physiological maturity, the plant head was excised and incubated in 40°C for three days. Kernel weight was, then, measured. Considering the two replications grown in the greenhouse as controls, the stress susceptibility index (SSI) of Fischer and Maurer (1978) was calculated as

$$SSI = \frac{1 - (Y_s / Y_p)}{SI} , \quad SI = 1 - \frac{\bar{Y}_s}{\bar{Y}_p}$$

Where Y<sub>s</sub> and Y<sub>p</sub> refer to the performance of each genotype in stress and control conditions, respectively, and  $\bar{Y}$  refers to the mean performance of all genotypes.

### Molecular Marker Analysis

DNA extraction was conducted through the method of Tai and Tanksley (1990). The marker genotype of 144 RILs was assessed with 166 wheat SSRs and 3 AFLPs. SSR primers were selected from several sets: GWM (Roder *et al.*, 1998), GDM (Pestsova *et al.*, 2000), and WMC (Gupta *et al.*, 2002). PCR was carried out in a MJ PTC-100 thermocycler (MJ Research, MA, USA) using the recommended temperatures for each primer pair. Sunrise<sup>TM</sup> 96 (GibCo BRL) and Sequi-Gen GT Sequencing Cell (BioRad) systems were used for electrophoresis on agarose and on polyacrylamide gels, respectively. The AFLP markers obtained according to Vos *et al.* (1995).

### Construction of the Map

Segregation distortion for all the loci was tested using a chi-square test. Markers deviating from the theoretical frequencies and one of the lines with more than 10% of the markers being heterozygous were excluded from the QTL data. For the construction of the genetic map, linkage analysis was performed using the program MAPMAKER (Lander *et al.*, 1987) and the Haldane mapping function (Haldane, 1919). After the removal of closely linked marker loci (<1 cM) the genetic map used for QTL mapping comprised 81 marker loci (997.4 cM) with an average marker density of 6.6 cM. This

covers 16 chromosomes of wheat including 1A, 1B, 2A, 2B, 2D, 3D, 4B, 4D, 5A, 5B, 5D, 6B, 6D, 7A, 7B and 7D.

### QTL Analysis

The QTL analysis was performed by the software package PLABQTL (Utz and Melchinger, 1996) based on composite interval mapping (CIM). Co-factors were assessed by the procedure cov SELECT. The threshold for the detection of a QTL was fixed at a LOD value of 2.0. The phenotypic variance of each QTL and of all detected QTLs were calculated through multiple regression.

## RESULTS AND DISCUSSION

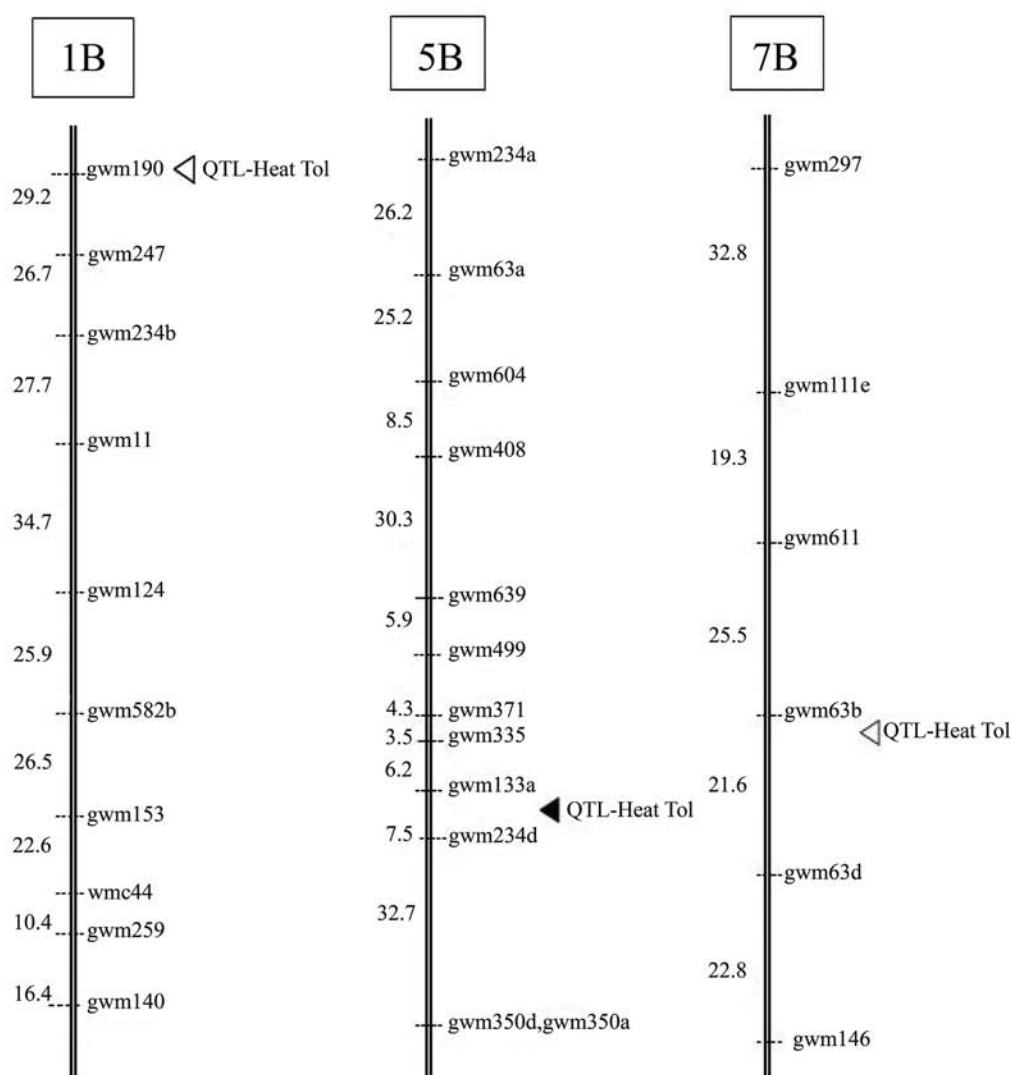
The kernel weight under stress conditions and SSI showed transgressive segregation (Table1) suggesting that the alleles of both parents contribute to heat tolerance and their combination results in higher values than those of the parents. The ANOVA showed that there is a significant variation among RILs for kernel weight under both stress and control conditions as well as for SSI. Heritability of SSI was fairly high indicating its reliability for QTL mapping. Despite the lack of statistical normality for SSI, we decided not to transform the data to achieve normality. As pointed out by Mutschler *et al.* (1996), transformation pulls the skewed tails of the distribution towards the center,

**Table 1.** Mean, range, Mean Square (MS), and heritability ( $h^2$ ) of the parents Kaz (KZ) and MTRWA116 (MT) and RILs derived from their cross for kernel weight (krnl wt) under stress and control conditions and their stress susceptibility index (SSI).

Trait	KZ	MT	Parent. Mean	RILs Min.	RILs Max.	RILs Mean	RILs MS	$h^2$
Control krnl wt	1.07	1.20	1.14	0.2	2.2	1.04	0.39**	0.53
Stress krnl wt	0.80	0.61	0.71	0.1	1.6	0.64	0.15*	0.20
SSI <sup>a</sup>	0.675	1.28	0.98	0.1	1.0	0.56	0.13**	0.67

\* and \*\* Significant at the 0.05 and 0.01 probability level, respectively.

<sup>a</sup> Stress intensity (SI) = 0.38.



**Figure 1.** Position of QTLs detected for heat tolerance on genetic map of 143 RILs derived from the cross Kauz × MTRWA116. White or black triangles indicate that allele for improved heat tolerance was inherited from Kauz or from MTRWA116

thereby misrepresenting differences in trait values among lines and reducing the ability to detect QTLs.

On the basis of composite interval mapping, three QTLs were detected for heat tolerance measured by SSI (Table 2). One of the QTLs is located on chromosome 1B and linked closely to gwm190 (Figure 1). This is a major QTL (LOD>3) and explains 44.3% of phenotypic variance. The positive additive effects of this QTL revealed that the allele from Kauz should be considered for

heat screening because the lower values of SSI are of interest. The second QTL located on chromosome 5B next to gwm133a explained 27.3% of the phenotypic variance. The negative additive effects of this locus demonstrates that MTRWA116 contributes the favorable allele for increased heat tolerance. This agrees with the results of transgressive segregation. For years, the group-5 chromosomes have been thought to carry genes for abiotic stress resistance (Dubcovsky *et al.*, 1995; Cattivelli *et al.*, 2002).

**Table 2.** QTLs for heat tolerance measured by stress susceptibility index for 143 RILs from the cross Kauz×MTRWA116 with the position on chromosome, the nearest marker, confidence interval, LOD score, explained phenotypic variance ( $R^2$ ), and additional effects.

Nearest marker	Position <sup>a</sup>	Confidence interval	LOD	$R^2$	Additive effects <sup>b</sup>
gwm190	1B(0)	0-14	3.43	44.3	0.131
gwm133A	5B(114)	112-132	2.01	27.3	-0.295
gwm63B	7B(78)	68-86	2.61	34	0.115
				16.7 <sup>c</sup>	

<sup>a</sup> The number in parenthesis shows the position on the chromosome of the QTL in cM.

<sup>b</sup> Negative effects indicate that the allele from MTRWA116 contributes to a higher heat tolerance.

<sup>c</sup> Total explained phenotypic variance in a simultaneous fit.

Another QTL is located on chromosome 7B and closely linked to gwm63b, having positive additive effects. Three QTLs altogether explained 16.7% of the phenotypic variance of SSI in a simultaneous fit which is much lower compared to the individual QTLs, suggesting a statistical correlation between QTLs.

Yang *et al.* (2002) reported two QTLs linked to gwm11 and gwm293 for heat tolerance which were not detected in our study. The reason for this might be that (1) they used grain filling duration rather than SSI for measuring heat tolerance, (2) the QTLs were detected in an F2 population passing only one meiosis and the recombination might, therefore, happen during the subsequent generations, or (3) these loci were possibly not segregating in our population. Interestingly, gwm11 was mapped on chromosome 1B where a QTL was detected for heat tolerance in this study (Figure1) indicating that, most probably, chromosome1B contains genes governing heat tolerance.

Since there was no similar study on QTL mapping for heat tolerance in the literature, comparison of detected QTLs with other studies was not possible.

A large amount of explained phenotypic variances and small confidence intervals indicated that the precision of the location of the QTLs detected was good enough and

that the linkage information between markers and QTLs could be used in breeding programs. Nonetheless, QTL analysis with a more saturated linkage map and more replications would add to the precision and reliability of the results.

## REFERENCES

1. Al-Khatib, K. and Paulsen, G. M. 1984. Mode of High Temperature Injury to Wheat during Grain Development. *Physiol. Plant*, **61**: 363-368.
2. Al-Khatib, K. and Paulsen, G. M. 1990. Photosynthesis and Productivity during High-Temperature Stress of Wheat Genotypes from Major World Regions. *Crop Sci.*, **30**: 1127- 1132.
3. Blum, A. 1988. *Plant Breeding for Stress Environments*. CRC Press. Boca Raton, FL.
4. Blum, A. and Nguyen, C. Y. 1997. The Effect of Plant Size on Wheat Response to Agents of Drought Stress. II. Water Deficit, Heat and ABA. *Plant Physiol.*, **24**: 35-41.
5. Butler, J. D., Byrne, P. F., Mohammadi, V., Chapman, P. L. and Haley, S. D. 2005. Agronomic Performance of Rht Alleles in a Spring Wheat Population across a Range of Moisture Levels. *Crop Sci.*, **45**: 939-947.
6. Cattivelli, L., Baldi, P., Cosatti, C., Di Fonzo, N., Faccioli, P., Grossi, M., Mastrangelo, A. M., Pecchioni, N. and Stanca, A. M. 2002. Chromosome Regions and Stress-Related Sequences Involved in Resistance to



- Abiotic Stress in Triticeae. *Plant Mol. Biol.*, **48**: 649-665.
7. Dubcovsky, J., Luo, M. C. and Devorak, J. 1995. Linkage Relationships among Stress-induced Genes in Wheat. *Theor. Appl. Genet.*, **91**: 795-801.
  8. Fischer, R. A. 1986. Physiological Limitations to Producing Wheat in Semitropical and Tropical Environments and Possible Selection Criteria. *Proc. Internal Symp. Wheat for Tropical Environments*. CIMMYT/UNDP, Mexico, pp. 209-230.
  9. Fischer, R. A. and Maurer, R. 1978. Drought Resistance in Spring Wheat Cultivars. I. Grain Yield Responses. *Aust. J. Agri. Res.*, **29**: 897 – 912.
  10. Fokar, M., Nguyen, T. and Blum A. 1998. Heat Tolerance in Spring Wheat. I. Estimating Cellular Thermotolerance and its Heritability. *Euphytica*, **104**: 1-8.
  11. Fokar, M., Nguyen, T. and Blum A. 1998. Heat Tolerance in Spring Wheat. II. Grain Filling. *Euphytica*, **104**: 9-15.
  12. Gupta, P. K., Balyan, H. S., Edwards, K. J., Isaac, P., Korzun, V., Roder, M., Gautier, M., Joudrier, P., Schlatter, A., Dubcovsky, J., De La Pena, R., Khairallah, M., Penner, G., Hayden, M., Sharp, P., Keller, B., Wang, R., Hardouin, J., Jack, P. and Leroy, P. 2002. Genetic Mapping of 66 New Microsatellite (SSR) Loci in Bread Wheat. *Theor. Appl. Genet.*, **105**: 413-422.
  13. Haldane, J. B. S. 1919. The Recombination of Linkage Values and the Calculation of Distance between the Loci of Linkage Factors. *J. Genet.*, **8**: 299-309.
  14. Iba, K. 2002. Acclimation Response to Temperature Stress in Higher Plants. *Annu. Plant. Biol.*, **53**: 225 - 245.
  15. Ibrahim, A. and Quick, J. S. 2001. Heritability of Heat Tolerance in Winter and Spring Wheat. *Crop Sci.*, **41**: 1401-1405.
  16. Jansen, E.C. 1993. Interval Mapping of Multiple Quantitative Trait Loci. *Genetics*, **135**: 205-211.
  17. Kato, K., Miura, H. and Sawada, S. 2000. Mapping QTLs Controlling Grain Yield and Its Components on Chromosome 5A of Wheat. *Theor. Appl. Genet.*, **101**: 1114-1121.
  18. Knott, S. A. and Haley, C. S. 1992. Maximum Likelihood Mapping of Quantitative Trait Loci Using Full-sib Families. *Genetics*, **132**: 1211-1222.
  19. Lander, E. S. and Botstein, D. 1989. Mapping Mendelian Factors Underlying Quantitative Traits Using RFLP Linkage Maps. *Genetics*, **121**: 185-199.
  20. Lander, E. S., Green, P., Abrahamson, J., Barlow, A., Daly, M. J., Lincoln, S. E. and Newberg, L. 1987. MAPMAKER- An Interactive Computer Package for Constructing Primary Genetic Linkage Maps of Experimental and Natural Populations. *Genomics*, **1**: 174-181.
  21. Liu, B. H. 1998. *Statistical Genomics; Linkage, Mapping and QTL Analysis*. CRC Press, LLC, USA.
  22. Maestri, E., Natalya, K., Perrotta, C., Gulli, M., Nguyen, H. and Marmioli, N. 2002. Molecular Genetics of Heat Tolerance and Heat Shock Proteins in Cereals. *Plant. Mol. Biol.*, **48**: 667-681.
  23. Mutschler, M. A., George, R. W. and Liu, S. C. 1996. QTL Analysis of Pest Resistance in the Wild Tomato; QTLs Controlling Acyl-sugar Level and Composition. *Theor. Appl. Genet.*, **92**: 709-718.
  24. Ozkan, H., Yagbasanlar, T. and Genc, T. 1998. Tolerance and Stability Studies on Durum Wheat Under Drought and Heat Stress Conditions. *Cereal. Res. Commun.*, **26**: 405-412.
  25. Pestsova, E., Ganal, M. W. and Roder M. S. 2000. Isolation and Mapping of Microsatellite Markers Specific for the D Genome of Bread Wheat. *Genome*, **43**: 689-697.
  26. Reynolds, M.P., Ortiz-Montasterio, J.I. and McNab, A. 2001. *Application of Physiology in Wheat Breeding*. CIMMYT, Mexico, 246 p.
  27. Reynolds, M. P., Balota, M., Delgado, M. I. B., Amani, I. and Fischer, R. A. 1994. Physiological and Morphological Traits Associated with Spring Wheat Yield under Hot, Irrigated Conditions. *Aust. J. Plant Physiol.*, **21**: 717-730.
  28. Roder, M. S., Korzun, V., Wendehake, K. 1998. A Microsatellite Map of Wheat. *Genetics*, **149**: 2007-2023.
  29. Sax, K. 1923. The Association of Size Differences with Seed Coat Pattern and Pigmentation in *Phaseolus vulgaris*. *Genetics*, **8**: 552- 560.
  30. Stuber, C. W., Edwards, M. D. and Wendel, J. F. 1987. Molecular Marker Facilitated Investigation of Quantitative Trait Loci in Maize. II. Factors Influencing Yield and Its Component Traits. *Crop Sci.*, **27**: 639-648.

31. Tai, T. and Tanksley, S. 1990. A Rapid and Inexpensive Method for Isolation of Total DNA from Dehydrated Plant Tissue. *Plant Mole. Biol. Report*, **8 (4)**: 297-303.
32. Utz, H. F. and Melchinger, A. E. 1996. PLABQTL- A Computer Program to Map QTL, Version 1.1. Institute Pflanzenzüchtung, Saatgutforschung und Populationsgenetik. Universität Hohenheim. Stuttgart. Germany.
33. Vos, P., Hogers, R. and Bleeker, M. 1995. AFLP- A new Technique for DNA Fingerprinting. *Nucleic acids Res.*, **23**: 4407-4414.
34. Wardlaw, I. F., Blumenthal, C., Larroque, O. and Wrigley, C. 2002. Contrasting Effects of Heat Stress and Heat Shock on Kernel Weight and Flour Quality in Wheat. *Funct. Plant. Boil.*, **29**: 25-34.
35. Wardlaw, I. F., Dawson, I. A. and Munibic, P. 1989. The Tolerance of Wheat to High Temperatures during Reproductive Growth. II. Grain Development. *Australian J. Agr. Research*, **40**: 1-13.
36. Yang, J., Sears, R. G., Gill, B. S. and Paulsen G. M. 2002. Quantitative and Molecular Characterization of Heat Tolerance in Hexaploid Wheat. *Euphytica*, **126**: 275-282.
37. Zeng, Z. B. 1994. Precision Mapping of Quantitative Trait Loci. *Genetics*, **136**: 1457-1468.

### نقشه یابی QTL های تحمل به گرما در گندم

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#### چکیده

تنش گرما یکی از مهمترین عوامل محدود کننده تولید گندم در مناطق خشک و نیمه خشک جهان به شمار می رود. تحمل گرما صفتی است کمی که ارزیابی فنوتیپی و تفکیک آن از خشکی بسیار مشکل می باشد. نقشه یابی QTL علاوه بر اطلاعات بسیار مفیدی که در زمینه جایگاه و تعداد ژنهای کنترل کننده فراهم می کند، می تواند بهنژادگران را در گزینش به کمک نشانگر یاری کند. به منظور نقشه یابی QTL های کنترل کننده تحمل گرما جمعیتی شامل ۱۴۴ لاین اینبرد نو ترکیب که از تلاقی واریته های کاز ومانتنا حاصل شده بودند، در گلخانه و اتاقک رشد با دمای ۳۵/۳۰ درجه سانتیگراد ارزیابی شدند و از شاخص فیشر به عنوان معیار تحمل گرما استفاده شد. نقشه لینکازی جمعیت با استفاده از ۱۶۶ نشانگر SSR و ۳ نشانگر AFLP ترسیم شد که هجده گروه لینکازی و شانزده کروموزوم گندم را پوشش می داد. تجزیه QTL به روش نقشه یابی بازه ای مرکب برای شاخص فیشر، سه QTL را برای تحمل گرما آشکار ساخت که روی کروموزومهای 1B، 5B و 7B قرار دارند. لازم به ذکر است که آللهای هر دو والد در بروز صفت نقش داشتند. ضرایب تبیین QTL ها بزرگ و بازه های اطمینان عموماً کوچک هستند که نشان می دهد از پیوستگی بین نشانگرها و QTL ها می توان در برنامه های اصلاحی استفاده کرد.